With Two Photon Microscopy (2PM) high definition images out of the depth of biological samples such as human skin can be obtained in a non-invasive way. Microscopic probe in this imaging method is the focus of an ultrashort pulsed laser being moved through the tissue, exciting endogeneous fluorophores in a nonlinear way. The autofluorescence photons induced by the nonlinear two-photon excitation are collected and used for imaging the scanned tissue.

With conventional Two Photon Microscopes the field of view is limited to areas of 500µm x 500µm at a resolution in the µm-range. Because diagnostic relevant imaging sizes in the medical field (for instance in dermatology) often exceed this range 2PM cannot be used as a diagnostical tool up to now.

Resulting out of our research a 2PM with a special scanning technique has been established, that overcomes this limits. Using this innovative technology scan areas of 10mm x 10mm x 0.5µm with a resolution up to 0.5µm can be realized. Using a “flying optics” approach not only laterally scanned images, but also vertical scanned images may be generated.

Synchronous recording of one to three images simultaneously and with exact local congruence, using photons from a choice of different spectral windows can merged into a false-colour presentation for interpretation according to a specific diagnostic question.

Current use of the 2PM ex vivo shows that it is appropriate for diagnosis and research applications of human skin tissue [1].

Figure 1: Imaging (vertical cut) of a specimen of human skin tissue in paraffin